

6. Kinetics and Drug stability



Introduction

- Stability
 - Refers to the extent to which the properties* of a drug substance or drug product remain within specified limits at certain temperature.
 - Or, the capacity of a drug product to remain within specifications established to ensure its identity, strength, quality, and purity.



- Importance of stability study
 - Chemical degradation results in a substantial loss of therapeutic agent in the dosage form.
 - Degradation products may result in adverse events or be unsafe.
 - Instability may cause:
 - Undesired change in performance, i.e. dissolution/bioavailability.
 - Substantial changes in physical appearance of the dosage form causing product failures.



- **Kinetics** is study of the rate at which processes or changes occur.
- The changes may be:
 - Chemical such as decomposition of a drug, radiochemical decay...
 - Physical such as transfer across a boundary, such as the intestinal lining or skin.



- The concept of Kinetics may be employed (examples):
 - The rate of physical changes e.g., viscosity changes;
 - The rate of kill of microorganisms by antimicrobial preservatives;
 - The rate of degradation of a drug substance or excipients;
 - The rate of transfer across a boundary, such as intestinal lining to blood.



- Pharmaceutical importance of studying kinetics:
 - To determine half life and shelf life
 - Selection of proper storage condition
 - Selection of proper container for dispensing
 - Anticipation of incompatibilities
 - Dissolution determinations
 - ADME Processes in pharmacokinetics

Rate and Order of Reactions

Rate

- Refers to the velocity or speed of a reaction with which a reactant(s) undergo a change.
- Can be measured by measuring the change in the concentration of a reactant or product in a particular period of time.
- Given by:

$$Rate = (\pm) \frac{dC}{dt} = kC^n$$

Where 'k' is the rate constant and 'n' is the order of a reaction

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Order of a reaction

- Refers to the way in which the concentration of the reactant(s) influence the rate of a chemical reaction.
- Example:
 - **Zero order reaction:** the rate of reaction is **independent** of the concentration of the reactants and constant with respect to time.
 - **First-order reaction:** the rate of reaction is **directly proportional** to the concentration of reactant remaining with respect to time.



Law of mass action

- The rate of a reaction is proportional to the product of the molar concentration of reactants each raised to power equal to the number of molecules undergoing reaction.
- That is, for the reaction:



- The rate can be given by: $\text{Rate} = k[A]^a[B]^b$
- The overall order = $a + b$
- k is the rate constant

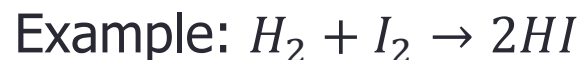
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Molecularity

- Refers to the number of molecules, ions or atoms in elementary process to give the product.
- **Unimolecular:** only one type of molecule undergoes a change to yield the product.



- **Bimolecular:** two molecule combine to give the product.





- Molecularity and order of a reaction are generally identical for elementary reaction.
- If a reaction proceeds through several stages (complex reactions), the term molecularity is not used in reference to the observed rate law.
- For the overall reaction, $2NO + O_2 \rightarrow 2NO_2$, the order has been found experimentally to be 2.
 - The mechanism is postulated to consist of two elementary steps, each being bimolecular ($2NO \rightarrow N_2O_2$ and $N_2O_2 + O_2 \rightarrow 2NO_2$)

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Specific rate constant (k)

- A constant associated with a single-step (elementary) reactions.
- Any change in the condition of the reaction will lead to rate law having a different value for k .
- Experimentally, a change of k corresponds simply to a change in the slope of the line given by the rate equation.

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Zero order reaction

- It is a reaction where reaction rate is not dependent on the concentration of the reacting species.
- Rate is constant and is not affected by changes in the concentration of any of the reactants.
- For the reaction/process: $A \rightarrow Product$

$$Rate = -\frac{dC}{dt} = k$$

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- From the above equation; $k = -\frac{dC}{dt}$, rearranging:

$$dC = -kdt$$

- Integrating this rate equation between the initial concentration (C_o) and concentration C after time t , will result in:

$$C = C_o - kt$$

- The plot of this linear equation (C vs t), the slope of the line gives $-k$.

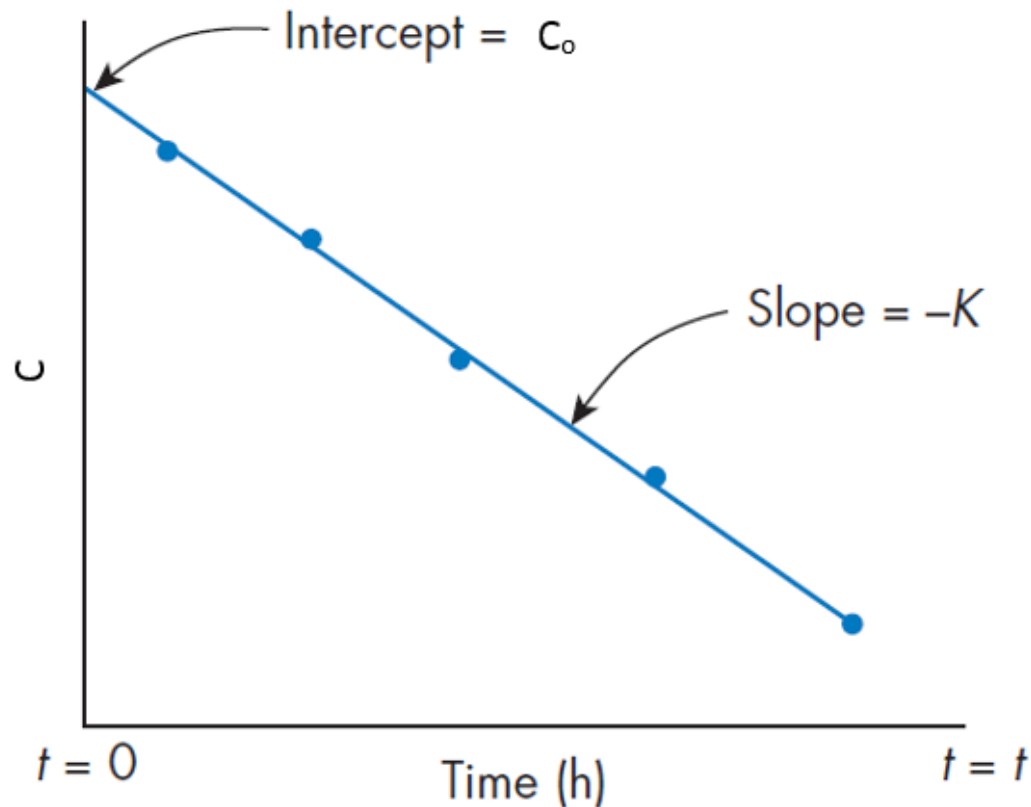


Fig. Zero-order process (X , concentration of drug; K , rate constant)

- The unit for the rate constant, k , in zero order reaction is ($\text{moles liter}^{-1} \text{ second}^{-1}$).



Half life of zero order reaction

- The half life is the time required for 50% of the material to disappear; it is the time at which C_o is decreased to $C_o/2$.
- At $t = t_{1/2}$, $C = C_o/2$, therefore, substituting these in the equation, $C = C_o - kt$, and rearranging:

$$t_{1/2} = \frac{C_o}{2k}$$

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Shelf life of a zero order reaction

- The shelf life is the time required for 10% of the material to disappear; it is the time at which C_0 has decreased to 90% of its original concentration.
- At $t = t_{0.9}$, $C = 0.9C_0$, therefore, substituting these in the equation, $C = C_0 - kt$, and rearranging:

$$t_{90\%} = t_{0.9} = \frac{0.1C_0}{k}$$



Example

- Drug X degrades by a zero-order process with a rate constant of 0.05 mg/ml/year at room temperature. If a 1 mg/ml solution is prepared and stored at room temperature;
 1. What concentration will remain after 18 months?
 2. What is the half-life and shelf-life of the drug?

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First-order reaction

- The rate depends on the concentration of one of a single reactant.
- In this type of reaction, the single reactant decomposes directly into one or more products;



With rate equation: $\frac{dC}{dt} = -kC$

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- Integrating the above equation between the limits of concentration C_0 at time $t=0$ and concentration C at $t=t$;

$$\int_{C_0}^C \frac{dC}{C} = -k \int_0^t dt$$

- With rearrangement, we get:

$$\ln C = \ln C_0 - kt$$

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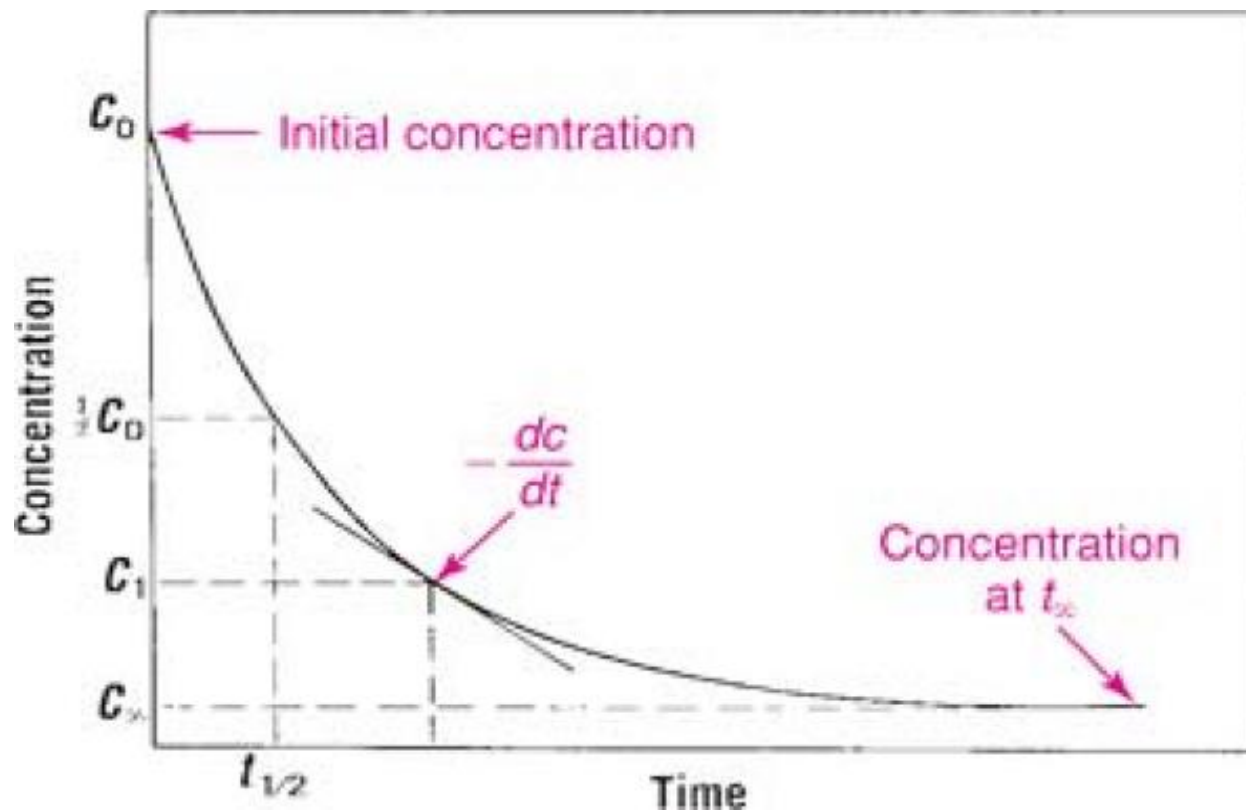
- This last equation can be expressed:
 - In exponential terms as

$$C = C_o e^{-kt}$$

- Or, in logarithmic form as

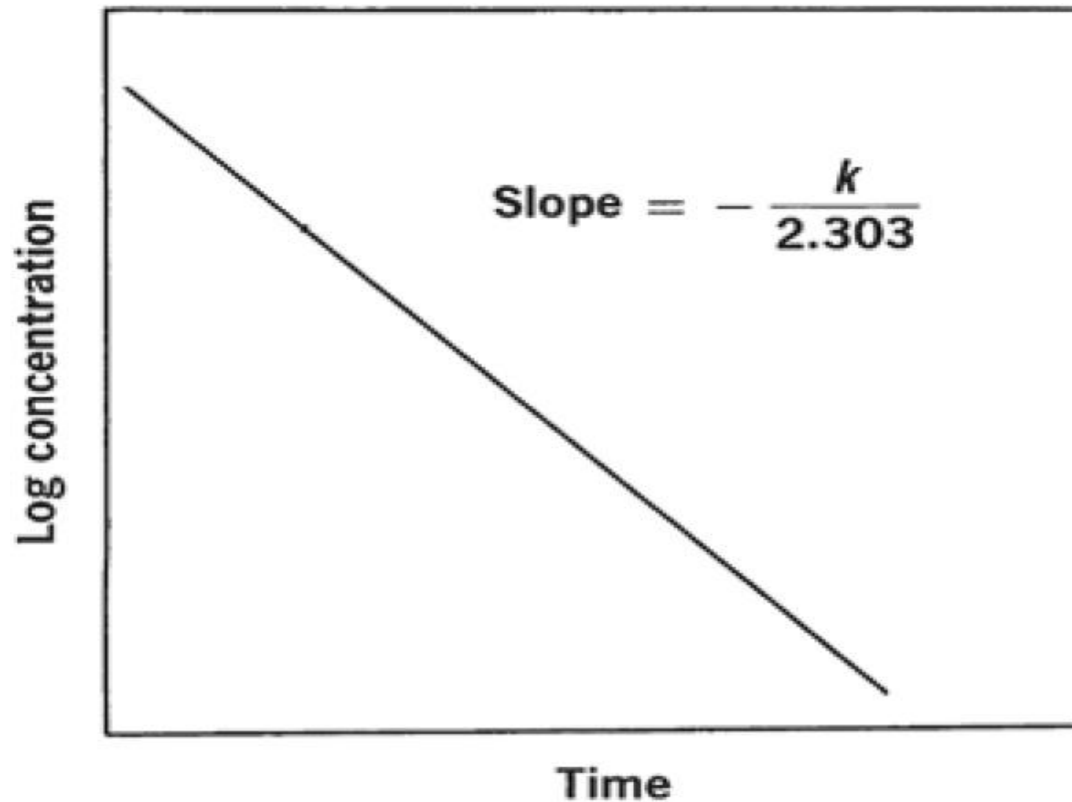
$$\log C = \log C_o - \frac{kt}{2.303}$$

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Concentration decrease exponentially with time (plot of C versus t).

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If $\log C$ is plotted against time t , a straight line is obtained with slope equal to $-k/2.303$.

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Half life of first order reaction

- From the equation: $\log C = \log C_0 - \frac{kt}{2.303}$, rearranging and solving for t,

$$t = \frac{2.303}{k} \log \frac{C_0}{C}$$

- At $t = t_{1/2}$, $C = C_0/2$, therefore, substituting these in the above equation,

$$t_{1/2} = \frac{0.693}{k}$$

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Shelf life of first order reaction

- At $t = t_{0.9}$, $C = 0.9C_0$, therefore, substituting these in the equation, $t = \frac{2.303}{k} \log \frac{C_0}{C}$:

$$t_{0.9} = \frac{0.105}{k}$$

- Note: the half life and shelf life of a first order reaction is a constant **independent** of the concentration



- Example

1. A solution of drug contained 680 units/ml when prepared. It was analyzed after a period of 60 days and was found to contain 400 units/ml. Assuming the decomposition is first order, at what time the drug have decomposed to one half of its original concentration?
2. What is the time taken for 5% of a drug to decompose by first-order kinetics ($k = 2.09 \times 10^{-5}$ per sec)?
3. The initial concentration of a drug which decomposes according to first order kinetics, is 94 units/ml. The specific decomposition rate ' k ' obtained is 2.09×10^{-5} per sec at 25 °C. Previous experiments have shown that below 45 units/ml, the drug is not fit for use. What expiry date should assigned to the product?

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Second-order reaction

- Rate of reaction is proportional either to the second power of the concentration of a single reactant or to the first power of the concentration of the two reactants.



- If the speed of the reaction depends on the concentrations of A and B with each term raised to the first power,

$$-\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A][B]$$

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- If a and b are the initial concentrations of A and B, respectively, and x is the concentration of each species reacting in time t , the reaction rate is written as:

$$\frac{dx}{dt} = k(a - x)(b - x)$$

where $a-x$ and $b-x$ are the concentrations of A and B, respectively, remaining at time t .

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- If both A and B present in the same concentration so that $a=b$ the equation is written as

$$\frac{dx}{dt} = k(a - x)^2$$

- Upon rearranging and integrated this equation,

$$\int_0^x \frac{dx}{(a - x)^2} = k \int_0^t dt$$

We get: $\left(\frac{1}{a-x}\right) - \left(\frac{1}{a-0}\right) = kt$ OR $\frac{x}{a(a-x)} = kt$

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- Solving for k:

$$k = \frac{1}{at} \left(\frac{x}{a-x} \right)$$

- Or, plot of $\frac{x}{a(a-x)}$ against t yields straight line and the slope of the line is k.

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- If A and B are not present in equal concentration, integration of equation yields:

$$\frac{2.303}{a-b} \log \frac{b(a-x)}{a(b-x)} = kt$$

- Solving for k:

$$k = \frac{2.303}{t(a-b)} \log \frac{b(a-x)}{a(b-x)}$$



- Or, plot of $\log \frac{b(a-x)}{a(b-x)}$ against t yields straight line and the slope of the line is $\frac{k(a-b)}{2.303}$.
- Note: The rate constant, k , in a second-order reaction has the dimensions liter/(mole sec) or liter mole⁻¹ sec⁻¹.

	Zero-order	First-order	Second-order	
			$a = b = c_0$	$a \neq b$
Differential rate expression	$-\frac{dc}{dt} = k$	$-\frac{dc}{dt} = kc$	$-\frac{dc}{dt} = kc^2$	$-\frac{dc}{dt} = kc_a c_b$
Integrated rate expression	$k = \frac{c_0 - c}{t}$	$k = \frac{1}{t} \ln \frac{c_0}{c}$	$\frac{1}{c} - \frac{1}{c_0} = kt$	$k = \frac{1}{t(a-b)} \ln \frac{b(a-x)}{a(b-x)}$
$t_{1/2}$	$\frac{c_0}{2k}$	$\frac{0.693}{k}$	$\frac{1}{c_0 k}$	(i) When $x = 0.5a$ $\frac{1}{k(a-b)} \ln \frac{0.5ab}{a(b-0.5a)}$ (ii) When $x = 0.5b$ $\frac{1}{k(a-b)} \ln \frac{b(a-0.5b)}{0.5ab}$
$t_{90\%}$	$\frac{c_0}{10k}$	$\frac{0.105}{k}$	$\frac{0.11}{c_0 k}$	(i) When $x = 0.1a$ $\frac{1}{k(a-b)} \ln \frac{0.9ab}{a(b-0.1a)}$ (ii) When $x = 0.1b$ $\frac{1}{k(a-b)} \ln \frac{b(a-0.1b)}{0.9ab}$

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Pseudo order reaction

- Apparent or pseudo order describes a situation where one of the reactants is present in large excess or does not affect the overall reaction and can be held constant.

Pseudo zero order reaction

- A compound decomposing in solution exhibits a first order reaction if more of it is present as insoluble excess, then an equilibrium is maintained between the compound in solution and that in the solid form.



- The concentration of the compound in solution, thus remains essentially constant till any of the insoluble drug material remains.
- Reaction taking place in pharmaceutical suspensions are generally of pseudo zero-order type.
- As the drug in solution decomposes more of it from the suspended particles goes into solution so that the concentration in the solution remains constant.



Pseudo First order reaction

- In second-order reaction, if the concentration of one reactant is in such large excess that it virtually remains constant, then the rate of change of concentration follows first-order.
- Hydrolysis reactions (acid or base catalyzed) is pseudo first order.
- If a buffer is used to maintain a desired pH, the reaction proceeding on addition of an acid or a base is pseudo first order.

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Methods for determining the order of reaction

- Substitution method
- Graphical method
- Half life method

Chemical and Physical Degradation

Chemical Degradation Routes

Hydrolysis (solvolysis)

- Interaction of drug molecules with water molecule to yield breakdown product (cleavage of bonds by action of water).
- Most important
 - In system containing water such as emulsion, suspensions, solutions.
 - For drugs which are affected by trace of moisture in the form of water vapor from the atmosphere.



- Protection against hydrolysis
 - Hydrolysis in solid dosage forms may be prevented by:
 - Avoiding their contact with moisture at the time of manufacture,
 - Packaging in suitable moisture resistant packs,
 - Storage in controlled humidity and temperature, and
 - Incorporating a suitable desiccant in the pack such as silica gel bags.



- Hydrolysis in liquid dosage form can be prevented by:
 - Choosing an optimum pH by inclusion of proper buffering agent,
 - Keeping buffer concentration to the minimum to reduce acid-base catalyzed hydrolytic decomposition,
 - Altering the dielectric constant of the system by partial or complete replacement of water,
 - Addition of specific complexing agent,
 - Preparing insoluble derivatives of the drug,
 - Formulating of dry powder for reconstitution or dispersible tablets,
 - Refrigeration of some drugs and drug solutions.

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Oxidation

- Usually mediated through reaction with atmospheric oxygen under ambient conditions.
- Involve multiple pathways for the initiation, propagation and termination.
- Many oxidation reactions are catalyzed by acids and bases.
- Often autoxidation reactions are initiated by trace amounts of impurities



- Protection against oxidation:
 - Antioxidants should be included in the formulation,
 - The oxygen in pharmaceutical containers should be replaced with nitrogen or carbon dioxide,
 - Avoiding contact of the drug with heavy-metal ions such as iron, cobalt or nickel, which catalyze oxidation,
 - Storage at reduced temperatures,
 - Oxidation of fats and oils may be retarded by hydrogenation.



Photodegradation

- Normal sunlight or room light may cause substantial degradation of drug molecules.
- The energy from light radiation may be absorbed by the molecules to cause a photolytic reaction.
- Primary photochemical reaction occurs when the wavelength of the incident light is within the wavelength range of absorption of the drug.



- Photolysis reactions often are associated with oxidation
 - Because the oxidation reactions frequently can be initiated by light.
 - But photolysis reactions are not restricted to oxidation.



- Protection against Photolysis
 - Use of colored glass containers (amber glass excludes light of wavelength < 470 nm),
 - Storage in the dark place,
 - Packaging in cartons (physical barrier to light),
 - Coating tablets with a polymer film containing UV absorbers.



Dehydration

- The elimination of a water molecule from structure of the compound.
- Anhydrous compounds may have different dissolution rates compared to their hydrates,
- Dehydration reactions involving water of crystallization may potentially affect the absorption rate of the dosage form.



Isomerization

- The process of conversion of a drug into its optical or geometric isomers.
- May be regarded as a form of degradation resulting in serious loss of therapeutic activity.
- Racemization and epimerization, which are reversible conversions between optical isomers, have been reported for many drug substances.



- Examples of isomerization of drug substance
 - Trans-cis isomerization of amphotericin B and vitamin A,
 - Pilocarpine undergoes epimerization by base catalysis,
 - Tetracyclines and Ergotamine exhibit epimerization by acid catalysis.
 - Epinephrine is oxidized and undergoes racemization under strongly acidic conditions.



Decarboxylation and Elimination

- Drug substances having a carboxylic acid group are sometimes susceptible to decarboxylation.
- Examples of drugs
 - 4-Aminosalicylic acid and Foscarnet under strongly acidic conditions.



Incompatibilities

- Chemical interactions between two or more drug components in the same dosage form or between active ingredient and a pharmaceutical adjuvant.
- E.g. the inactivation of cationic aminoglycoside antibiotics, such as kanamycin and gentamycin, by anionic penicillin in IV admixtures

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Physical Degradation Routes

Vaporization

- Some drugs and excipients possess sufficiently high vapor pressures at room temperature that their volatilization constitutes a major route of drug loss.
- Flavors, whose constituents are mainly ketones, aldehydes, and esters, and co-solvents (LMW alcohols) may be lost from the formulation.



- Reduction of vapor pressure be achieved through dispersion of the volatile drug in macromolecules that can provide physicochemical interactions.
- The addition of macromolecules such as polyethylene glycol, polyvinylpyrrolidone, and microcrystalline cellulose allows for preparation of “stabilized” nitroglycerin sublingual tablets



Aging

- Changes in the disintegration and/or dissolution characteristics of the dosage forms.
- The melting time of aminophylline suppositories, prepared from various bases, increased from about 20 minutes to over an hour after 24 weeks of storage at 22 °C.
- Thus, an increase in melting time can perceivably lead to a decrease in bioavailability.



Adsorption (Sorption)

- Drug-plastic interaction is a major potential problem when IV solutions are stored in bags or infused via administration sets that are made from PVC.
- Up to 50% drug loss can occur after nitroglycerin is stored in PVC infusion bags for 7 days at room temperature.
- More than 40% of a dose of quinidine gluconate was lost when the drug was administered with a conventional PVC IV administration set.



Crystallization of Amorphous Drugs

- Poorly water-soluble drugs formulated in their amorphous state to improve the solubility.
- Amorphous substances tend to change to their more thermodynamically stable crystalline state with time.
- This may occur during long-term storage and may lead to drastic changes in the release characteristics of the drug.



Transitions in Crystalline States

- Polymorphic transitions during storage may alter critical properties of drugs.
- Polymorphs are different crystalline forms of the same drug.
- Temperature and humidity affect polymorphic transitions.



Formation and Growth of Crystals

- Molecules in a crystal, and the crystals themselves, should not be considered static.
- Crystals can grow or decrease in size provided that there is a medium across which the molecules can travel.



Factors affecting stability of drugs:

- Environmental factors: Temperature, Light, Oxygen, Moisture and Carbon dioxide,
- Drug/excipients in the dosage form: Particle size of drug, pH of the vehicle,
- Contamination: Microbial, Trace metal
- Leaching from containers
- Formulation factors: Processing method, Mixing/Millings

Stability study

- **Purpose:**
 - To determine **quality** of a drug product and ensure a stable product on the market.
 - To provide evidence on how the quality of a drug/drug product varies with time under the influence of a variety of environmental factors.
 - To establish a **retest** period for the drug substance or a **shelf-life** for the drug product and recommended **storage** conditions



- Stability ~ a product in a specific container/closure system to remain within its specifications:
 - Physical,
 - Chemical,
 - Microbiological,
 - Therapeutic, and
 - Toxicological



- Stability studies should include testing of those attributes of the drug/drug product that are susceptible to change during storage and are likely to influence quality, safety, and or efficacy.
- The testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes, preservative content and functionality test.



- Potential instability issues:
 - Loss/increase in concentration of API,
 - Formation of (toxic) degradation products,
 - Alteration of dissolution time/profile or bioavailability
 - Decline of microbiological status
 - Loss of package integrity
 - Loss of pharmaceutical elegance and patient acceptability ...



- There are three types of stability studies:
 - Chemical stability
 - Physical stability
 - Microbiological stability

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Chemical stability

- Chemical stability implies the lack of any decomposition in the chemical moiety that is incorporated in the formulation as the drug, preservatives or any other excipients.
- Decomposition may influence the physical and chemical stability of the drug.
- Chemical instability may be caused by oxidation, hydrolysis and photolysis.



Physical stability

- Physical stability implies that the formulation has not suffered any changes by way of appearance, organoleptic properties, hardness, brittleness, particle size etc.
- It is significant as it affects
 - Pharmaceutical elegance
 - Drug content uniformity
 - Drug release rate



- Likely physical instability problems:
 - For oral solutions: Instability may change the smell, feel or taste of the preparation
 - Loss/change of flavor, Change in taste, Loss of dye, Precipitation, Discoloration.
 - For parenteral preparations: Instability may change the appearance and bio-availability
 - Discoloration due to photo chemical reaction or oxidation
 - Presence of precipitate due to interaction with container or stopper
 - Clouds



- For suspensions: Instability may cause the loss of drug content uniformity in different doses from the bottle and loss of elegance
 - Settling, Caking, Crystal growth
- For emulsion: Instability may cause the loss of drug content uniformity in different doses from the bottle and loss of elegance
 - Creaming, Coalescence
- For semisolids: Instability may cause the loss of drug content uniformity in different doses from the bottle, loss of elegance and a change in drug release rate
 - Changes in Particle size and Consistency, Caking or coalescence



- For tablets: Instability may cause change in drug release
 - Change in disintegration time, Change in dissolution profile, Change in hardness, Change in appearance (soft and ugly or become very hard).
- For capsule: Instability may cause change in drug release
 - Strength, Dissolution profile, Appearance

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Microbiological stability

- Implies that
 - The formulation has not suffered from any microbiological attack and,
 - The formulation meeting the standards with respect to lack of contamination/sterility.



Zoning and stability study protocols

- The world has been divided in to four zones taking in to account the prevalent annual climatic conditions of temperature and humidity (ICH and WHO guideline)

Zone I – Temperate

Zone II – Subtropical (with possible high humidity)

Zone III – Hot, dry

Zone IV – Hot, humid



- Major types of stability tests:
 - Long term stability test
 - Field stability test
 - Accelerated stability test
- These tests involve application of a suitable **stress** or **challenge** such as temperature, light, RH, gravity, and microorganism, over a period of time and assess its effects.

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Long term stability (Real time studies)

- Long term studies are defined as stability studies under the recommended storage conditions for the shelf life proposed or approved for labeling.
- Conducted in the laboratory under controlled stresses similar to those likely to be encountered during storage.
- 12 months minimum and conducted at 25°C ($\pm 2^\circ\text{C}$) and at a relative humidity of 60% ($\pm 5\%$).

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Field stability test

- Done at manufacturers laboratory after the packaged drug product has transported to a typical pharmacy level and stored at different levels.



Accelerated stability study

- Use of exaggerated conditions of temperature, humidity, light & others.
- Accelerated storage condition
 - 6 months study at 40 °C (± 2 °C) with 75% ($\pm 5\%$) relative humidity.



- Accelerated stability tests are done:
 - To detect the deterioration of the product rapidly, in order to select the best formulation;
 - To predict shelf life;
 - To provide rapid means of quality control.



Testing frequency

- The frequency of at the long term storage condition should be every 3 months over the first year, every 6 month over the second year and annually thereafter through the proposed shelf life.
- At the accelerated storage condition, a minimum of three points, including the initial and final time points (e.g. 0, 3 and 6 months), from the six month study, is recommended.



Stability testing protocols

- Accelerated stability testing requires a careful design of protocols which must clearly define:
 - The temperature and humidity for storage,
 - Storage time before sampling,
 - The number of batches to be sampled,
 - The number of replicates within each batch,
 - A suitable light challenge,
 - Details of assay.

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Prediction of shelf life

- Shelf life – the time period during which the dosage form is supposed to retain its original qualities.



- Steps involved in prediction of shelf life:
 - The preparation is divided in to different portions and each portion is stored at different temperatures,
 - Samples from each portion are withdrawn at various time intervals and the remaining concentration of the active ingredients is measured,
 - The order of reaction is determined by a suitable methods such as graphic, substitution method, or half-life,
 - From the slopes of the lines, the reaction rate constant k for the degradation at each of the elevated temperatures is calculated.



- A similar method with some modifications has also been suggested for the determination of shelf life:
 - Log percent remaining is plotted against time (in days),
 - From the graph, the time required for the potency to fall to 90% of the original value at different temperature is obtained,
 - Then the log time to 90% is plotted against the reciprocal of temperature,
 - The straight line obtained is extrapolated to lower temperature (25) and from this the number of days required for the potency to fall to 90% of its original value is read directly at 25.



- Stability at room temperature for drug products recommended for cold storage.
- It is not always possible to attain the recommended temperature.
- Drug products may be incorrectly stored for different reasons:
 - Refrigeration units may fail;
 - Drug products may be left out of the refrigerator
 - Goods may be stored in transit at an undesirable temperature

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- To quantitatively estimate the changes in chemical stability due to temperature , Q_{10} concept is used.
- Q_{10} value is the factor by which a rate constant increase for a 10 °C rise in temperature;
- Read more ...

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Expiration Date

- The date placed on the immediate container label of a drug product that designates the date through which the product is expected to remain within specifications.
- Read more ...
 - Shelf-life Vs Expiry date??